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**BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES**

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*Ex parte*
RICHARD F. SELDEN, ALLAN M. MILLER, and DOUGLAS A. TRECO

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Appeal 2007-3217
Application 09/686,497
Technology Center 1600

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DECIDED: January 25, 2008

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Before TONI R. SCHEINER, DONALD E. ADAMS, and RICHARD M. LEBOVITZ, *Administrative Patent Judges*.

SCHEINER, *Administrative Patent Judge*.

DECISION ON APPEAL

Appellants appeal under 35 U.S.C. § 134 from a final rejection of claims 1-14 and 26-31, all the claims remaining in the application, as obvious over the prior art. We have jurisdiction under 35 U.S.C. § 6(b).

We affirm.

## BACKGROUND

"An approach to increasing protein yield using recombinant DNA technology is to modify the coding sequence of a protein of interest . . . without altering the amino acid sequence of the gene product" (Spec. 2-3). This approach involves altering the native gene sequence "such that codons which are not so frequently used in mammalian cells are replaced with codons which are overrepresented in highly expressed mammalian genes" (Spec. 3).

## STATEMENT OF THE CASE

The present invention is directed to an "optimized" synthetic nucleic acid sequence encoding human α-galactosidase, wherein at least one native codon, which is not the codon most frequently represented in humans for a given amino acid, is replaced by the most frequently represented codon for that amino acid.

Claims 1-14 and 26-32 stand rejected under 35 U.S.C. § 103(a) as unpatentable over Seed,[1] Kim,[2] Morgan,[3] Bishop,[4] and Wada.[5]

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[1] International Patent Application WO 96/09378 of Brian Seed, published March 28, 1996.
[2] Chang H. Kim at al., *Codon Optimization for High-Level Expression of Human Erythropoietin (EPO) in Mammalian Cells*, 199 Gene 293-301 (1997).
[3] Stephen H. Morgan et al., *Anderson-Fabry Disease - Family Linkage Studies Using Two Polymorphic X-Linked DNA Probes*, 1 Pediatr. Nephrol. 536-553 (1987).
[4] David F. Bishop et al., *Human α-Galactosidase A: Nucleotide Sequence of a cDNA Clone Encoding the Mature Receptor*, 83 Proc. Natl. Acad. Sci. USA 4859-4863 (1986).
[5] Ken-nosuke Wada et al., *Codon Usage Tabulated from the Genbank Genetic Sequence Data*, 20 Nucleic Acids Research 2111-2118 (1992).

Appellants have not argued the rejected claims separately. Therefore, we select claim 1 as representative of the claimed subject matter for the purpose of deciding this appeal, and claims 2-14 and 26-32 will stand or fall with claim 1. 37 C.F.R. § 41.37(c)(1)(vii).

Claim 1 reads as follows:

> 1. A synthetic nucleic acid sequence that encodes human α-galactosidase, wherein at least one non-common codon or less-common codon has been replaced by a common codon and wherein the synthetic nucleic acid has one or more of the following properties:
>
> it has a continuous stretch of at least 150 codons all of which are common codons;
>
> it has a continuous stretch of common codons, which continuous stretch comprises at least 60% of the codons of the synthetic nucleic acid sequence;
>
> at least 94% of the codons in the sequence encoding the protein are common codons, wherein by a common codon is meant Ala (gcc); Arg (cgc); Asn (aac); Asp (gac); Cys (tgc); Gln (cag); Gly (ggc); His (cac); Ile (atc); Leu (ctg); Lys (aag); Pro (ccc); Phe (ttc); Ser (agc); Thr (acc); Tyr (tac); Glu (gag); Val (gtg); Met (atg) and Trp (tgg).

According to the Specification, the common codons listed in claim 1 are "the most common codon[s] representing a particular amino acid in a human sequence"[6] (Spec. 21). "'Less-common codons' are codons that occur frequently in humans but are not the common codon: Gly (ggg), Ile (att); Leu ([c]tc); Ser (tcc), Val (gtc); and Arg (agg). All codons other than common codons and less-common codons are 'non-common codons'" (*id.*).

[6] Methionine (Met) and tryptophan (Trp), of course, are each represented by a single codon.

## FINDINGS OF FACT[7]

*Kim*

1. "The level of gene expression of eukaryotic genes introduced into mammalian cells depends on various factors such as gene copy number, transcriptional control elements, the site of chromosomal integration, mRNA stability and translational efficiency" (Kim 293, left-hand col.).

2. "[C]ontrol of gene expression at the translational levels is mostly governed by the coding gene structure" (Kim 293, right-hand col.).

3. One "way to increase the protein yield is to modify the coding sequence of an individual gene without altering the amino acid sequence of the gene product" (Kim 294, left-hand col.).

4. "[T]he choice of synonymous codons in many species is strongly biased and . . . a correlation exists between high expression and the use of selective codons in a given organism" (Kim 294, left-hand col.), that is, "codon-optimized gene[s]" are efficiently expressed (Kim 294, left-hand col.).

5. "Re-engineering the coding sequence to match the codons frequently found in human genes is beneficial to achieve high-level expression" (Kim 299, right-hand col.) in mammalian cells.

6. "[H]uman prevalent codons usually have C or G at their third degenerative position . . . [t]hus, sequence engineering with human codon usage can result in stable mRNA secondary structures because of stronger GC base pairing" (Kim 294, left-hand col.).

7. Kim's human "prevalent" codons are the same as Appellants' common codons recited in claim 1 (Kim Fig. 1).

[7] Abbreviated "FF".

8. Kim "designed two human erythropoietin (EPO) genes, one in which native codons were systematically substituted with codons frequently found in highly expressed human genes and the other with codons prevalent in yeast genes" (Kim Abstract; Fig. 2).

9. In 293T cells (human embryonic kidney cells), "the expression plasmid containing the EPO gene with the human high frequency codon ($EPO^h$) directed the synthesis of EPO more efficiently than the plasmid with the yeast prevalent codon-based EPO gene ($EPO^y$), shown by their expression levels, which were 37.2 and 14.7 U/ml, respectively" (Kim 297, right-hand col.), relative to EPO units produced using the reference recombinant EPO control gene (Kim 297, left-hand col.).

10. "[A] given gene optimized with human codon usage becomes high in GC content" and "it is known that mRNA with a high GC content of the 5' untranslated region (*UTR*) may be translated with low efficiency" (Kim 298, right-hand col.), thus, decreasing the GC content of 5'-*UTR*, and "the limited region downstream of the initiator codon of the . . . gene could result in an increased [protein] expression" (*id.*).

*Seed*

11. Seed describes "a method for preparing a synthetic gene encoding a protein normally expressed by mammalian cells. The method includes identifying non-preferred and less-preferred codons in the natural gene encoding the protein and replacing one or more of the non-preferred and less-preferred codons with a preferred codon encoding the same amino acid as the replaced codons" (Seed 3: 8-14).

12. "Preferred codons are: Ala (gcc); Arg (cgc); Asn (aac); Asp (gac); Cys (tgc); Gln (cag); Gly (ggc); His (cac); Ile (atc); Leu (ctg); Lys (aag); Pro (ccc); Phe (ttc); Ser (agc); Thr (acc); Tyr (tac); . . . Val (gtg)" (Seed 1: 27-30), and Glu (gag) (*id.* at 9: 18-20).

13. Seed's "preferred," "non-preferred," and less-preferred" codons are the same as Appellants' "common," "non-common," and "less-common" codons.

14. A "protein normally expressed in mammalian cells is . . . a protein which is expressed in mammalian [cells] under natural conditions. The term includes genes in the mammalian genome such as Factor VIII, Factor IX, interleukins, and . . . also includes genes . . . which are encoded by a virus (including a retrovirus) which are expressed in mammalian cells post-infection" (Seed 2: 1-9).

15. "In order to produce a gp120 gene capable of high level expression in mammalian cells, a synthetic gene encoding the gp120 segment of HIV-1 was constructed . . . [in which] nearly all of the native codons have been systematically replaced with codons most frequently used in highly expressed human genes" (Seed 10: 19-27). "[T]he synthetic gene product is expressed at a very high level compared to that of the native gp120 controls" (Seed 12: 26-27; Fig. 3) in human cell lines.

16. Seed teaches "[u]nder some circumstances (e.g., to permit introduction of a restriction site) it may be desirable to replace a non-preferred codon with a less-preferred codon rather than a preferred codon" (Seed 3: 15-18). Also, "it may be desirable to avoid CpG sequences as these sequences may cause gene silencing" (Seed 4: 11-12).

*Wada*

17. Wada lists the common codons for a number of species, including humans (Wada 2112, Table 1).

*Bishop and Morgan*

18. "Anderson-Fabry disease is an X-linked lysosomal storage disorder due to α-galactosidase A deficiency. In affected males there is a high mortality in early adult life" (Morgan, Abstract).

19. "The complete nucleotide sequence has been determined for a . . . cDNA clone . . . containing the full-length coding region for the mature lysosomal form of human α-galactosidase A" (Bishop, Abstract), and the predicted amino acid sequence of the enzyme was collinear with a microsequenced portion of the purified mature enzyme (*id.*).

*The Claimed Invention*

20. The mature form of human α-galactosidase contains approximately 398 amino acids (Bishop Abstract).

21. Claim 1 is directed to a synthetic nucleic acid sequence encoding human α-galactosidase, wherein at least one non-common or less-common codon in the native α-galactosidase sequence has been replaced by a human common codon, and wherein the synthetic sequence has a continuous stretch of 150 common codons, *or* a continuous stretch of common codons that comprises at least 60% of the synthetic sequence, *or* at least 94% of the codons in the synthetic sequence are common codons.

DISCUSSION

The question of obviousness is resolved on the basis of underlying factual determinations including: (1) the scope and content of the prior art; (2) the level of ordinary skill in the art; (3) the differences between the claimed invention and the prior art; and (4) secondary considerations of nonobviousness, if any. *Graham v. John Deere Co.*, 383 U.S. 1, 17-18 (1966). "When there is a design need or market pressure to solve a problem and there are a finite number of identified, predictable solutions, a person of ordinary skill has good reason to pursue the known options within his or her technical grasp. If this leads to the anticipated success, it is likely the product not of innovation but of ordinary skill and common sense. In that instance the fact that a combination was obvious to try might show that is was obvious under § 103." *KSR Int'l Co. v. Teleflex Inc.*, 127 S.Ct. 1727, 1742 (2007).

Essentially, the Examiner contends that it would have been obvious for one of ordinary skill in the art "to develop a method for making α-galactosidase" because Morgan and Bishop teach that Anderson-Fabry disease is due to a deficiency of α-galactosidase (Answer 4), and it would have been obvious to enhance the production of α-galactosidase in mammalian cell culture by optimizing the native human gene by replacing the "less-common" and "non-common" codons with human "common codons," because Seed and Kim teach that optimized genes are expressed more efficiently than non-optimized genes, and Wada lists all the common human codons (Answer 4-5).

Appellants argue that "the references, singly and in combination, fail to suggest any gene, much less the specifically claimed gene (human α-Gal), having the high levels of common codons required by the claims" (Br. 4). In addition, Appellants argue that "the references provide a pattern of teaching away from such high levels of optimization of a human gene" (*id.*). Finally, Appellants argue that "the nucleic acids of the claims have surprising and unexpected properties" (*id.*).

According to Appellants, "the native human α-galactosidase sequence has a much lower level of human preferred codons than the [optimized] synthetic sequence recited in the claims" (Br. 6), and both Seed and Kim teach away from "optimizing at the recited levels" (*id.* at 7). In particular, Appellants argue that "[o]ne of ordinary skill would have been expressly discouraged by Seed from replacing the codons of . . . genes with human common codons" (*id.* at 5), because "[t]he use of common codons . . . increases the number of CpG pairs" (*id.*), and Seed teaches that "it may be desirable to avoid CpG sequences as these sequences may cause gene silencing" (Seed 4: 10-12; Br. 4). Similarly, Appellants argue that Kim suggests that further optimizing a human re-engineered gene "*by decreasing the GC content* of the limited region downstream of the initiator codon *is advisable*" (Br. 8; Kim 299). Thus, Appellants argue that Kim, "by *cautioning against the overuse of human common codons*, continues the pattern of teaching away seen in the . . . Seed reference" (Br. 8).

First of all, we disagree with Appellants' assertion that either Seed or Kim teaches away from replacing most of the non-common and less-common codons in a human gene with common human codons. A reference

is said to "teach away" from a claimed invention when it "suggests that the line of development flowing from the reference's disclosure is unlikely to be productive of the result sought by the applicant." *In re Gurley*, 27 F.3d 551, 553 (Fed. Cir. 1994). That is not the case here.

Seed does teach that it may be desirable to replace a non-preferred codon with a less-preferred codon, rather than a preferred codon, under limited circumstances, for example, to introduce a restriction site. Seed also teaches that CpG sequences may cause gene silencing (Seed 3: 15-18; 4: 11-12; FF 16). Nevertheless, Seed went on to produce a synthetic gp120 gene, highly expressed in human cell lines, in which "*nearly all* of the native codons . . . [were] systematically replaced with codons most frequently used in highly expressed human genes" (Seed 3: 15-18; 4:11-12; 10: 19-27 (emphasis added); FF 15, 16). Similarly, Kim produces a synthetic EPO gene in which non-common codons "were systematically substituted with codons frequently found in highly expressed human genes" and merely cautions against a high GC content in the 5'*UTR* and "the *limited region* downstream of the initiator codon" (Kim Abstract; Fig. 2; 298, right-hand col. (emphasis added); FF 8, 10). We find that neither reference would lead one skilled in the art to expect that systematically replacing "nearly all" of the non-common codons in a natural sequence with human common codons would be unlikely to produce a synthetic gene expressed at higher levels than the natural gene.

Moreover, we note that a synthetic α-galactosidase gene with a continuous stretch of as few as 150 common codons (only about 38% of the

sequence encoding human α-galactosidase) would meet the limitations of claim 1 (FF 19, 20).

Appellants also argue that Kim "does not refer to optimization with *human* high-frequency codons. Instead, the hybrid EPO gene described by Kim contained *yeast* high-frequency codons *at the 5' leader sequence and at the sequence encoding the first six amino acids* of mature EPO, and *human* high frequency codons throughout most, but not all, of the rest of the EPO hybrid gene" (Br. 8).

This argument is without merit. Kim explicitly teaches that the synthetic EPO gene systematically substituted with human high frequency codons was expressed more efficiently than the recombinant EPO control gene (Kim 297; FF 9). That Kim was able to further enhance expression of the synthetic gene by replacing non-common codons in the limited region downstream of the initiator codon with yeast high frequency codons in no way detracts from that fact. Moreover, as discussed above, one could replace non-common codons in a similarly limited region with yeast high frequency codons, or even leave the non-common codons in place, and still meet the limitations of claim 1.

Finally, Appellants argue that "synthetic α-galactosidase sequences containing the much larger continuous stretches or overall very high numbers of common codons recited in the claims, resulted in 2.0 and 5.7-fold increases in mean α-galactosidase expression compared to the wild-type sequence" (Br. 6), and that "[t]hese are surprising results, especially in view of the teaching away by Seed and Kim" (*id.*).

This argument is not persuasive. As discussed above, we do not agree that either Seed or Kim teaches away from the claimed invention. Moreover, Appellants have not shown that the prior art optimized genes have shorter continuous stretches than that required by the claims (e.g., a continuous stretch of at least 150 codons all of which are common codons, *or* a continuous stretch of common codons, which continuous stretch comprises at least 60% of the codons of the synthetic nucleic acid sequence (Claim 1)). Finally, Appellants have not established that a 2-fold, or even 5.7-fold increase in expression would have been unexpected given Seed's and Kim's results (FF 9, 15).

We conclude that the Examiner has established a prima facie case that the invention of claim 1 would have been obvious over the combined teachings of the cited prior art, which Appellants have not overcome by argument or evidence. As discussed above, claims 2-14 and 26-31 stand or fall accordingly. The Examiner's rejection of claims 1-14 and 26-32 as unpatentable over Seed, Kim, Morgan, Bishop, and Wada is affirmed.

## CONCLUSION

We affirm the rejection of claims 1-14 and 26-32 as unpatentable under 35 U.S.C. § 103(a).

No time period for taking any subsequent action in connection with this appeal may be extended under 37 C.F.R. § 1.136(a).

AFFIRMED

Ssc:

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